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# A fermented nutraceutical beverage from quinoa: The traditional grain of Andes

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Quinoa is a traditional grain used as staple food by ancient people of Andean countries and South America, known for its excellent nutritional profile. This pseudo-cereal is rich in protein, vitamins, minerals and antioxidants. However, presence of anti-nutritional factors such as, saponins, tannins, phenols and phytic acid is a serious deterrent to use quinoa in daily diet. This study focuses on lactic acid fermentation to answer this riddle. Lactic acid bacteria were isolated from the surface of quinoa seeds and characterized. All the isolates (QB-1, QB-2, QB-3, QB-4 and QB-5) were Gram positive, Catalase negative, acid producing and non-endospore forming. Two among the native isolates, QB-1 and QB-2 were selected based on their ability for beverage development. Isolate QB-1 reduced the phenolic compounds (from 0.94 to 0.36 mg/g of GAE) significantly. Isolate QB-2 was found to be more efficient in reduction of phytic acid (from 11.06 to 1.00 mg/g) and tannins (from 4.92 to 2.05 mg/g of GAE). A significant reduction of saponin (from 11.2 to 0.13 mg/g) was recorded by isolate QB-1 and *Lactobacillus delbrueckii*. The study also revealed that, fermentation with curd is also efficient in reducing anti-nutritional factors such as, phenolic compounds and phytic acid.

**Keywords**: Anti-nutritional factors, Fermentation, Lactic acid bacteria, Nutraceutical beverage, Quinoa

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United Nations has declared the year 2013 as the "International Year of Quinoa" in recognition of the indigenous people of Andes. The traditional practices of Andean people managed to preserve this pseudo-cereal in its natural form as a food[1]. In the past, we have witnessed the hailing of a series of abandoned crops such as kiwi fruit, soy bean and oil palm into globally significant crops. Quinoa (*Chenopodium quinoa* Wild) is one of them. It is a pseudo-cereal belonging to the family Chenopodiaceae. It has gained worldwide attention being an underutilized crop due to its nutritional value especially high protein and dietary fiber profile[2]. Moreover, the seed quality of quinoa is not depleted considerably while growing in the salt affected soil[3]. This shows the significance of this crop in the present scenario of soil depletion. On an average, protein content in quinoa ranges from 8 to 22% which is significantly higher than that of common food grains such as rice and wheat[4]. Total dietary fiber content is 7% - 9.7% (on par with that in grain products) and while the soluble fiber content is in the range of 1.3 - 6.1%. The main carbohydrate component of quinoa is starch, constitutes 52% - 69% of the grain[5]. This underutilized crop is reported to have a higher content of Iron as compared to the traditional grains. Ash content of quinoa is 3.4%, whereas, rice and wheat show only 0.5% and 1.8% respectively. Moreover, calcium and iron content of quinoa is quantified to be considerably higher than that of other commonly used grains. Magnesium content in quinoa is 0.26% wherein, wheat and corn have only 0.16% and 0.14%, respectively. The excellent composition of calcium, magnesium and potassium in quinoa seeds are sufficient for a balanced diet[6].

Quinoa is discovered to be rich in nicotinic acid and α- carotenoids. The seeds contain an appreciable amount of folic acid, thiamin and vitamin C. As compared to ragi and barley, the vitamin profile of quinoa is substantially high with respect to riboflavin, carotene and α- tocopherol. Quinoa contains good amount of pantothenic acid, vitamin $B_6$, folic acid, biotin and ascorbic acid[3]. Polysaccharide fractions, both water and alkali-extractable (two each) were identified from seeds of quinoa. These polysaccharides not only comprise of natural antioxidants for health promotion but also, serve as functional foods or nutraceutical ingredients to modify immune system[7]. In a series of experiments on rats, quinoa seeds are found to act as a moderate protective agent against fructose-induced changes. This

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is because, quinoa reduce lipid peroxidation and also enhance the antioxidant capacity of blood (plasma), heart, testis, lungs, kidney and pancreas[8]. Moreover, regular consumption of quinoa has found to have potential ability to modify incremental area under blood glucose response curve[9]. Interestingly, the pseudocereal showed potential activity in reduction of level of glucose (10%), serum total cholesterol (26%), LDL and triglycerides in the blood of rats[10].

Being a pseudocereal with high nutritional value, gluten-free content and therapeutic features, quinoa benefit high-risk group consumers including, the elderly, children, people with lactose intolerance, people with anemia, high-performance sports people, women prone to osteoporosis, diabetes, dyslipidemia, celiac disease and obesity[11]. Malted quinoa beverage had antidiabetic and antihypertensive potential. Hence, it can be effectively included in diet[12].

However, quinoa is not only rich in nutrients but anti-nutritional factors such as saponins, phenolics, tannins and phytic acids. The primary anti-nutritional factor in quinoa is saponin which has pharmacological properties but imparts bitter taste as well as allergy. This reduces palatability of quinoa. Phytic acid is evenly distributed in quinoa endosperm and outer layers and these are responsible for the unavailability of minerals. Moreover, quinoa contains trypsin inhibitor as well as protease inhibitor in small concentrations[2]. So, overcoming these negative impacts of quinoa is the need of the hour for a better utilization. From eras ago, fermentation has various applications in food preservation. Thus, fermentation is used in various industrial processes by application of microorganisms including lactic acid bacteria and yeast. Lactic acid bacteria (LAB) are being used in food industries and for beverage fermentation over thousands of years due to the wide range of benefits that they add to food products. LAB improves food product quality by enhancing their shelf-life, safety, organoleptic and textural properties, as well as providing health benefits to the consumers[13]. The strain of *Lactobacillus plantarum* Q823 was successfully identified as a potential probiotic bacterium for its use as a starter culture in the fermentation of a quinoa-based beverage[14]. The probiotic strain of *Lactobacillus casei* shows good viability ($10^8$ CFU·mL$^{-1}$) in symbiotic formulation composed of soy and quinoa[15]. Various strains of *Lactobacillus acidophilus* and *L. plantarum* exhibited phytase activity as they were grown on calcium fortified soymilk supplemented with potassium phytate[16].

Though studies have been conducted for developing fermented beverages from quinoa, scanty published research on reduction of anti-nutritional factors in quinoa using fermentation is available. The present investigation attempted to develop a nutraceutical fermented beverage from quinoa, focusing mainly on reducing anti-nutritional factors. The study also utilizes common techniques such as germination in combination with fermentation to remove undesirable components from quinoa. Moreover, in this study, curd is also used as an inoculum for making this technique available for common people.

## Materials and Methods

### Samples and chemicals

The proven authenticated cultures of *Lactobacillus acidophilus* (MTCC-10307) and *Lactobacillus casei* MTCC 5303 were procured from Microbial Type Culture Collection Center (MTCC), Chandigarh, India. *Lactobacillus delbrueckii* (NCIM No. 5356) was purchased from NCIM, Pune. These proven cultures were used for fermentation studies. Quinoa seeds were procured from a farmer in Ranchi (Jharkhand) and used for enumeration and isolation of lactic acid bacteria and further fermentation studies. Other chemicals used for the study were, de Man, Rogosa and Sharpe (MRS) Agar, crystal violet, Gram's iodine, Ethyl alcohol, malachite green, safranine, sucrose, agar, hydrogen peroxide, Tributyrin agar, Sodium bicarbonate, and Folin-Ciocalteu reagent.

### Isolation of lactic acid bacteria

Lactic acid bacteria were isolated from quinoa surface by slightly modified enrichment method[17] using sterilized milk to enrich natural lactic acid bacteria. The isolates obtained on MRS agar medium[18] by pour plate method, colonies grown on subsurface transferred to MRS broth and incubated. The broth was again plated out on MRS agar medium and more isolated colonies were obtained. Then the dominant colonies were grown in Mannitol Salt Agar[19] to identify and discard *Staphylococci*. Remaining isolates were further subjected to Gram staining and catalase test after purification. Colonies with Gram positive and catalase negative characteristics were selected for further purification.

### Characterization of lactic acid bacterial isolates

Lactic acid bacterial isolates were observed for their colony characteristics on MRS agar medium, growth in MRS broth and cell shape were observed under

microscope. Lactic acid bacterial isolates (24 h) were stained with crystal violet, observed under microscope and their shapes were recorded. Gram staining[20] and endospore staining[21] were conducted as per standard procedures. The isolates were streaked on sucrose agar plates[22] and observed for dextran production after three days of incubation. Catalase activity test was performed according to a previous work[23]. Gelatin hydrolysis was performed according to the methods described by Ewing, 1962[24]. Nutrient gelatin stabs were prepared; stabs were inoculated with each culture and incubated at room temperature for 4 days. After incubation, tubes were placed in refrigerator at 4°C for 15 min. Liquefied nutrient gelatin tubes were recorded as gelatinase positive and those tubes that remained in solid state were recorded as gelatinase negative. Starch hydrolysis, casein hydrolysis and lipid hydrolysis were carried out using the same procedure used in previous work[25]. Lactic acid bacterial isolates were tested for acid and gas production. They were inoculated into test tubes having lactose broth containing Durham's tube in an inverted position and phenol red as pH indicator. The tubes were incubated for 72 h at room temperature and observed for colour change and gas accumulation in Durham's tube[26]. Methyl Red and Voges-Proskauers (MRVP) test was performed by following the same procedure of previous works[27].

#### Development of quinoa fermented beverage

Quinoa seeds were germinated, roasted and powdered. Standard protocol was prepared using the results of various experiments conducted for the preparation of quinoa fermented beverage using probiotic organisms (*Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus delbrueckii*, curd (procured from local market) and lactic acid bacterial isolate QB-1 and QB-2. Five percentage quinoa powder was used for production of beverage[28]. All treatments were incubated at 30°C for 4 days. After incubation, beverage was filtered, pasteurized at 60°C for 30 min and stored at 4°C in refrigerator. Utilization of indigenous techniques such as germination and fermentation for reducing antinutritional factors is the highlight and invention in this study. Curd being an easily available consortium of lactic acid bacteria is also used for fermentation, so that common people will have an easy access to this technique.

### Biochemical analysis of fermented beverage

Phenolic compounds in the quinoa fermented beverage were determined by Fazeli *et al.* method[29]. Tannins were determined using Folin-Ciocalteu method. Phytic acid contents in the fermented samples were determined by modified colorimetric method[30]. Saponin content in the sample was determined by method described by Obdoni and Ochuko[31].

## Results and Discussion

Microbial load on quinoa surface were assessed by enumeration. The results (Fig. 1) show that, quinoa is rich in bacteria, out of that a small proportion also constitutes lactic acid bacteria. The contribution of moulds and yeast to the microbial population is very less as 7.1 and 4.8 x $10^2$ cfu/ mg of sample. This may be due to substrate depletion by the rapid growth and high population of bacteria, might have retarded the growth of yeasts and molds present on the substrate[32]. These results are in concurrence with experimental results reported in the past[33]. The lactic acid bacteria, isolated from quinoa surface (enriched with sterilized milk for 24 h) were preserved in MRS slants. The isolates (eight) showed distinguishable characteristics were grown on mannitol salt agar. Isolates (five in number) were selected from mannitol salt agar and subjected for microscopic observation and biochemical analysis. The isolates were either surface or subsurface colonies with a creamy white or pure white colour. Most of the cells were observed as diplococci or cocci under microscope. Similar results were reported previously[34].

Biochemical characterization shown that, all the isolates were Gram positive, non-spore forming and showed negative results to catalase activity as shown in Table 1. *Lactobacillus* is already known to be catalase negative, gram positive, non-spore forming and prefer microaerophilic conditions over aerobic conditions[35]. Isolates QB-1, QB-2 and QB-5 were able to hydrolyze starch whereas QB-3 and QB-4 showed no starch hydrolysis. All the lactic acid

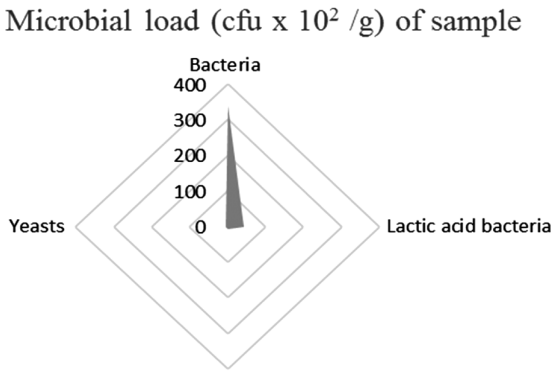


Fig. 1 — Microbial population on quinoa seed surface

Table 1 — Biochemical characterization of lactic acid bacterial isolates of quinoa

| Biochemical Test | QB-1 | QB-2 | QB-3 | QB-4 | QB-5 |
|---|---|---|---|---|---|
| Gram reaction | + | + | + | + | + |
| Starch hydrolysis | + | + | - | - | + |
| Dextran production | - | - | - | - | - |
| Gelatin hydrolysis | - | - | - | - | - |
| Endospore staining | - | - | - | - | - |
| MR/VP test | +/- | +/- | +/- | +/- | +/- |
| Catalase activity | - | - | - | - | - |
| Acid production | + | + | + | + | + |
| Gas production | - | - | - | - | - |
| Casein hydrolysis | - | - | - | - | - |
| Exopolysaccharide Production | - | - | - | - | - |
| Lipid hydrolysis | - | - | - | - | - |

+: Present, - : Absent

Table 2 — Sensory characteristics of beverage fermented with different isolates of quinoa (average of ten members)

| Treatments | Appearance (2) | Color (2) | Aroma (2) | Bouquet (2) | Vinegar (2) | Total acidity (2) | Sweetness (1) | Body (1) | Flavor (2) | Astringency (2) | General quality (2) | Overall acceptability (20) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $T_1$ | 1.18 | 1.28 | 1.84 | 1.73 | 1.76 | 1.67 | 0.45 | 0.84 | 1.82 | 1.64 | 1.74 | 18.10 |
| $T_2$ | 1.12 | 1.12 | 1.56 | 1.67 | 1.43 | 1.54 | 0.54 | 0.75 | 1.73 | 1.53 | 1.67 | 17.87 |
| $T_3$ | 1.23 | 1.45 | 1.67 | 1.43 | 1.37 | 1.65 | 0.67 | 0.56 | 1.54 | 1.34 | 1.23 | 15.12 |
| $T_4$ | 1.09 | 1.23 | 1.68 | 1.56 | 1.89 | 1.45 | 0.34 | 0.67 | 1.71 | 1.54 | 1.56 | 17.17 |
| $T_5$ | 1.03 | 1.43 | 0.93 | 1.32 | 1.23 | 0.98 | 0.75 | 0.34 | 1.11 | 1.28 | 1.10 | 14.13 |
| $T_6$ | 1.34 | 1.25 | 1.78 | 1.68 | 1.87 | 1.62 | 0.35 | 0.78 | 1.79 | 1.61 | 1.70 | 17.90 |

Note: $T_1$ : QB-1, $T_2$: QB-2, $T_3$: QB-3, $T_4$ : QB-4,$T_5$ :QB-5, $T_6$ : *Lactobacillus delbrueckii*

bacterial isolates failed to produce dextran from sucrose even after three days of incubation. MRVP test indicated that, the isolated organisms perform mixed acid fermentation pathway and no butanediol fermentation. All the isolates were detected with acid production and no isolates produced gas. The selected five isolates did not exhibit casein hydrolysis, exopolysaccharide production and lipid hydrolysis.

The beverage was fermented with five isolates, using reference organism *Lactobacillus delbrueckii*. The results reveal that, among the five isolates, QB-1 (Plate 1) was the most acceptable one with better taste (overall acceptability 18.1 out of 20). Table 2 and Table 3 depict the results of sensory evaluation and biochemical analysis of fermented quinoa drink. The pH of the beverages was significantly differing with all the treatments. The pH reduction was the highest (3.97) in beverage fermented with QB-1 followed by *Lactobacillus delbrueckii* (4.04). The least reduction in pH was recorded by QB-5 (4.61). The pH was reduced considerably in all the treatments after fermentation. The highest Titrable acidity (TA) was recorded with the beverage fermented using isolate QB-1 and *Lactobacillus delbrueckii* (0.61%) followed by QB-2 (0.60%). The isolates QB-1 and QB-2 were capable of fermenting quinoa very efficiently. These

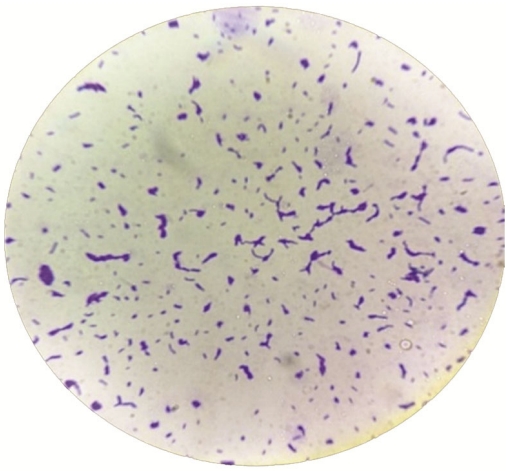

Plate 1 — Microscopic observation of isolate QB-1

isolates, QB-1 and QB-2 were selected for further fermentation studies due to its favorable sensory attributes and efficiency of fermentation.

Fermented quinoa beverage was prepared using the standard protocol using various organisms viz., *Lactobacillus acidophilus*, *Lactobacillus delbrueckii*, *Lactobacillus casei*, curd and the two proven isolates (QB-1 and QB-2). The developed beverage (Plate 2) was subjected to analysis for estimating antinutritional factors namely, phenolic compounds, tannins, phytic

Table 3 — Titrable acidity and pH of beverages fermented with different isolates of quinoa

| Treatment details | | pH | | Titrable acidity | |
|---|---|---|---|---|---|
| Treatments | Isolates | Initial | Final | Initial | Final |
| $T_1$ | QB-1 | 6.15 | 3.97[a] | 0.03 | 0.61[a] |
| $T_2$ | QB-2 | 6.14 | 4.12[b] | 0.02 | 0.60[a] |
| $T_3$ | QB-3 | 6.14 | 4.21[c] | 0.02 | 0.42[b] |
| $T_4$ | QB-4 | 6.15 | 4.38[d] | 0.03 | 0.39[b] |
| $T_5$ | QB-5 | 6.15 | 4.61[e] | 0.02 | 0.32[c] |
| $T_6$ | *L. delbrueckii* | 6.15 | 4.04 | 0.02 | 0.61[a] |

Table 4 — Antinutritional factors of quinoa beverage

| Treatments | Inoculum | Tannins (mg/g of gallic acid equivalent | Phenols (mg/g of gallic acid equivalent | Phytic acid (mg/g of sample) | Saponins (mg/g of sample) |
|---|---|---|---|---|---|
| Raw seed powder (before germination) | | 4.92[a] | 0.91[a] | 11.06[a] | 11.2[a] |
| Raw seed powder (after germination) | | 4.23[b] | 0.94[b] | 7.23[b] | 3.2[b] |
| $T_1$ | QB-1 | 3.47[e] | 0.36[f] | 2.01[f] | 0.13[d] |
| $T_2$ | QB-2 | 2.05[h] | 0.53[d] | 1.00[g] | 0.18[cd] |
| $T_3$ | *L. acidophilus* | 2.94[f] | 0.54[d] | 6.03[c] | 0.15[cd] |
| $T_4$ | *L. delbrueckii* | 2.45[g] | 0.66[c] | 3.02[e] | 0.13[d] |
| $T_5$ | *L. casei* | 3.90[c] | 0.68[c] | 4.02[d] | 0.19[c] |
| $T_6$ | Curd | 3.54[d] | 0.47[e] | 2.01[f] | 0.14[cd] |

Note: Substrates for treatments $T_1$, $T_2$, $T_3$, $T_4$, $T_5$ and $T_6$ were germinated, roasted and powdered.

acid and saponins. Results showing change in the amount of anti-nutritional factors of quinoa are presented in Table 4.

A study conducted by Nishitani *et al.* [36] showed that, different lactic acid bacterial species have the ability to degrade tannins from food products. In the present study of fermentation of quinoa, quantity of tannin was significantly influenced by fermentation. Estimated tannin content in raw seed and germinated seed powder before fermentation was 4.92 and 4.23 mg/g of gallic acid equalent (GAE). Tannin content of fermented beverage ranges between 2.05 to 3.90. The lactic acid bacterial isolate QB-2 recorded with highest reduction in the tannin content (2.05 mg/g of GAE) followed by *Lactobacillus delbrueckii* (2.45 mg/g of GAE). The least reduction in tannins was recorded in beverage fermented with *Lactobacillus casei* (3.90 mg/g of GAE) followed by curd (3.54). Lactic acid bacteria could bring a significant reduction in tannin content because, they are able to hydrolyze low molecular tannins as well as complex tannins[37].

Cariochi *et al.*[38] studied the effect of malting on phenolic compounds of quinoa and found that, germination and roasting increased the phenolic compounds and antioxidants present in quinoa seeds. Similarly, in the present study, total phenolic compounds increased after germination (from 0.91 to


Plate 2 — Fermented beverage of quinoa

0.94). A drastic reduction in phenolic compounds was recorded after fermentation. The amount of phenolic compounds in fermented beverage varies between 0.36 to 0.68 mg/g of GAE. In contrast to reduction of tannins, QB-1 was found to be more efficient in reduction of phenolic compounds (0.36 mg/g of GAE) followed by curd (0.47 mg/g of GAE). Germination could reduce the amount of phenolic compounds significantly. The amount of phenolic compounds

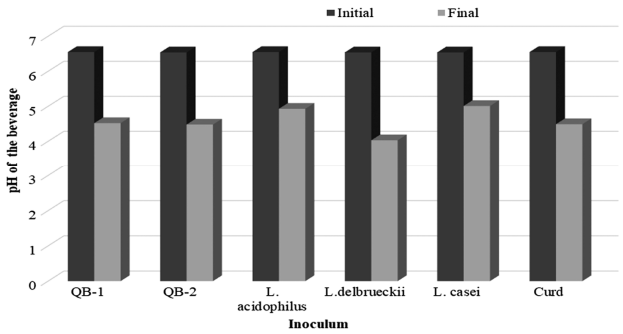


Fig. 2 — pH of fermented quinoa beverage

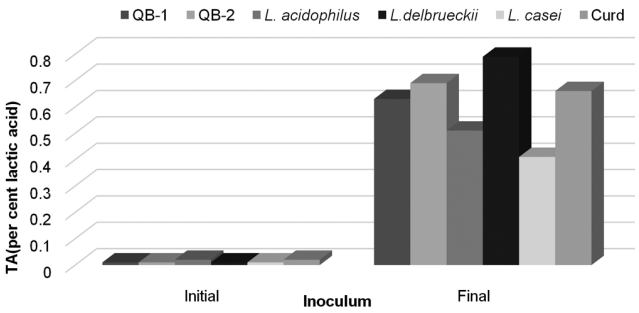


Fig. 3 — Titrable acidity of fermented quinoa beverage

in fermented beverages varied between 0.36 to 0.68 mg/g of GAE. The least reduction in phenolic compounds was estimated in beverage fermented with *L. casei* (from 0.94 to 0.68 mg/g of GAE).

A significant reduction in amount of phytic acid was found after germination as well as fermentation. Phytic acid was reduced from 11.06 to 7.23 mg/g after germination. Fermentation further reduced the amount of phytic acid to a range of 6.03 to 1.00 mg/g of sample. Isolate QB-2 was found to be more efficient in reduction of phytic acid (1.00 mg/g) followed by curd and isolate QB-1 (2.01). A reduction in phenolic compounds and saponins content in beverages was observed after fermentation. Saponin content reduced drastically after fermentation. Saponin content in the raw quinoa seed powder was 11.2 mg/g of sample. Washing and germination reduced saponins content to 3.20 mg/g of sample. This is because, majority of saponins are present in the outer layers of quinoa. So, washing and thorough rinsing in water can remove significant amount of saponins[39]. Similarly, reduced saponins content was recorded after germination in a previous study[40]. The saponin content of fermented beverages ranged between 0.13 to 0.19 mg/g of sample. Isolate QB-1 and *Lactobacillus delbrueckii* were found to be more efficient in reduction of saponin (0.13 mg/g of sample).

Other biochemical attributes such as pH and titrable acidity of the developed products were analyzed. Highest reduction in the pH (Fig. 2) was observed with *Lactobacillus delbrueckii* (4.03) followed by beverage fermented with curd (4.49) and QB-1 (4.48). The least reduction in pH was recorded in beverage fermented with *L. casei* (from 6.54 to 5.01). Titrable acidity of the treatments varied between 0.41 and 0.79. *Lactobacillus delbrueckii* has efficiently increased the titrable acidity from 0.01 to 0.79 followed by QB-2 (from 0.01 to 0.69). The least reduction in titrable acidity was recorded in the beverage fermented with *L. casei* (Fig. 3).

## Conclusion

Quinoa, being a pseudo- cereal and an underutilized crop, is rich with nutritional properties. However, detrimental about this cereal is the presence of antinutritional factors like saponins, tannins, phytates and phenols. The present study attempted to resolve this problem using traditional techniques such as fermentation and germination. The study proved that, antinutritional factors in quinoa can be reduced by germination and subsequent fermentation of the seeds. The lactic acid bacterial isolates of quinoa were proved to be effective in improving the acceptability of quinoa as well as reducing most of the antinutritional factors present in it. Germination also reduced saponin and phytic acid in quinoa. Moreover, sensory attributes and reduction in antinutritional factors in quinoa beverage fermented with curd was found to be comparable to that of isolates. This paves the path for bringing this extremely nutritious beverage on common men's table and popularizing it up to house hold level.

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## Conflict of Interest

The authors report no conflict of interest.

## Authors' Contributions

SKS conducted the experiments and drafted the manuscript. SVC provided necessary guidance and critical inputs in correcting the manuscript. SP and VKV helped in review collection and editing the manuscript.

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